Genetic structure of the high dispersal Atlanto-Mediterreanean sea star Astropecten aranciacus revealed by mitochondrial DNA sequences and microsatellite loci

Zulliger, D E ; Tanner, S ; Ruch, M ; Ribi, G

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ORIGINAL PAPER

Genetic structure of the high dispersal Atlanto-Mediterreanean sea star *Astropecten aranciacus* revealed by mitochondrial DNA sequences and microsatellite loci

Deborah E. Zulliger · S. Tanner · M. Ruch · G. Ribi

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D. E. Zulliger and S. Tanner contributed equally to this manuscript.

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D. E. Zulliger (✉) · S. Tanner · M. Ruch · G. Ribi
Zoological Museum of the University of Zurich,
Winterthurerstrasse 190, 8057 Zurich, Switzerland
e-mail: deborah.zulliger@access.unizh.ch

Introduction

Marine species with extended planktonic larval stages have a high capacity for dispersal and as such are expected to display less genetic structure than species without a long stage in the plankton (Palumbi and Wilson 1990). Nevertheless, gene-flow in marine species can be constrained by dispersal barriers, such as narrow water passages between land masses, sharp salinity gradients or different types of currents e.g., circular currents (eddies) or downward currents. As marine barriers are not always easily identified, they might lead to population structure even in high dispersal species (Quesada et al. 1995; Palumbi et al. 1997).

The role of the Atlantic–Mediterranean division as a potential barrier to gene-flow has increasingly been investigated for various planktotrophic invertebrate species (e.g., Borsa et al. 1997; Launey et al. 2002; Diaz-Almela et al. 2004; Duran et al. 2004a; Stamatis et al. 2004, 2006; Saavedra and Pena 2005; Calderon et al. 2008). While the Strait of Gibraltar geographically divides the two basins, the Almería-Oran front is thought to be a genetic separation area. This large-scale density front is formed by the convergence of two distinct water masses in the east Alboran Sea, which is located in the westernmost region of the Mediterranean Sea (Tintore et al. 1988). Other barriers to gene-flow

within the Mediterranean may also exist in the form of an east–west divide at the Siculo-Tunisian Strait and/or hydrogeographic isolation of the Aegean, Ionian and Adriatic Seas (Perez-Losada et al. 2007).

Many studies have used indirect genetic tools such as mitochondrial DNA (mtDNA), nuclear DNA or a combination of the two to analyze genetic structure in high dispersal Atlanto-Mediterranean invertebrates (e.g., Féral et al. 1995; Zane et al. 2000; Launey et al. 2002; Diaz-Almela et al. 2004; Duran et al. 2004a; Roman and Palumbi 2004; Stamatis et al. 2004; Triantafyllidis et al. 2005; Peijnenburg et al. 2006; Calderon et al. 2008). Differing conclusions regarding the influence of the Atlantic–Mediterranean division on population structuring were drawn in these studies depending not only on the species investigated but also on the genetic markers used and the sampling pattern. For instance, only moderate genetic differentiation was revealed between Atlantic and Mediterranean populations of the sea urchin *Paracentrotus lividus* based on mtDNA sequences (Duran et al. 2004a), whereas a sharp break was detected between the two basins when combining mitochondrial and nuclear markers and applying a more extensive sampling (Calderon et al. 2008). Based on allozymes and 28S rRNA sequence data, a clear separation was also detected in the sea urchin *Echinocardium cordatum* (Féral et al. 1995). Patarnello et al. (2007) discovered that even between closely related taxa with comparable biologies the Atlanto-Mediterranean transition does not always induce a congruent population genetic structure, which could be due to the differences in demographic history between these species. Restriction fragment length polymorphism (RFLP) of mtDNA in the Norway lobster *Nephrops norvegicus*, for example, showed no genetic differentiation between the Atlantic and the Mediterranean (Stamatis et al. 2004). However, pronounced differentiation between Atlantic and Mediterranean populations was detected in the closely related European lobster *Homarus gammarus* (Triantafyllidis et al. 2005) using also RFLP of mtDNA. Two other crustaceans, the high dispersal green crab *Carcinus maenas* (Roman and Palumbi 2004) and the pelagic Northern krill *Megenyctiphanes norvegica* (Zane et al. 2000), again showed genetic differentiation between the basins based on mtDNA sequences.

Gene-flow in marine species with long planktonic larval stages may be more restricted than generally assumed. In such cases, random drift occurs locally, and genetic structure can develop in the form of isolation-by-distance. This genetic pattern has been revealed in some high dispersal marine invertebrates, such as in the European flat oyster *Ostrea edulis* using microsatellite loci and mtDNA sequence data (Launey et al. 2002; Diaz-Almela et al. 2004) and in the pelagic crustacean *Megenyctiphanes norvegica* (Zane et al. 2000) using mtDNA data only.

Here we present data from another high dispersal Atlanto-Mediterranean echinoderm: the sand star *A. arranciacus*. *A. aranciacus* is a broadcast spawning sea star, which undergoes a long planktotrophic larval stage. Due to its large body size of up to 60 cm in diameter and the potential for high population densities, it is believed to be an important benthic predator (Burla et al. 1976). In the Mediterranean, this sea star was once abundant (Burla et al. 1972). However, over the past 20 years a decline in populations of *A. aranciacus* has been observed in several areas within the Mediterranean (G. Ribi unpublished; H. Lessios, H. Massé, H. Moosleitner, L. Santella, personal communications). The present distribution of *A. aranciacus* includes the Mediterranean Sea and the east Atlantic coast from northern Portugal to Angola, including the Canary Islands, Cape Verde and Madeira (e.g., Koehler 1921; Tortonese 1980). This sea star usually lives in depths of 1–100 m (Zavodnik 1960), but has been found at depths of up to 183 m (Hörstadius 1938). Migration of adult sea stars is therefore bound to the continental shelf and does not tend to occur in any particular direction (Pabst 1986); hence, adult movements are likely to be only a minor factor for dispersal. In southern Portugal, *A. aranciacus* is still highly abundant (C. Almeida, personal communication). With a planktonic larval stage of up to 60 days (Hörstadius 1938), *A. aranciacus* larvae can likely disperse up to 400 km following the calculations of Shanks et al. (2003). This high potential for dispersal might allow populations in the east Atlantic, such as for instance from southern Portugal, to replenish the Mediterranean populations along the prevailing water exchange direction, if there are no barriers to larval dispersal between these two basins.

While sea stars have been subject to several population genetic studies using mitochondrial and/or nuclear markers (Hunt 1993; Williams 2000; Williams and Benzie 1997, 1998; Williams et al. 2002; Matsuoka and Asano 2003; Waters et al. 2004; Waters and Roy 2004; Colgan et al. 2005; Harper and Hart 2005; Harley et al. 2006; Harper et al. 2007; Gerard et al. 2008), only one study was conducted in the Atlanto-Mediterranean region (Baus et al. 2005). This study found high genetic structure between Atlantic and Mediterranean populations of *Asterina gibbosa*, a sea star which is expected to have a low dispersal capacity, as it lacks a planktotrophic larval stage.

The present study investigates potential marine barriers to gene-flow in a high dispersal marine invertebrate by analyzing the population genetic structure of *A. aranciacus* employing both mitochondrial and nuclear markers. The comparison of these two marker types allows a more comprehensive investigation of genetic diversity, as markers of these two physically unlinked genomes do not always show congruent patterns (e.g., Hansen et al. 1999; Lemaire et al. 2005; Costantini et al. 2007). In this study, we sequenced

the complete control region of the mitochondrial DNA, a region that has the highest rate of evolutionary change of any mtDNA region (Aquadro and Greenberg 1983; Parsons et al. 1997). As nuclear markers, we used four polymorphic microsatellite loci, which are believed to be neutral and have been shown to be more variable compared to e.g., allozyme data (Shaw et al. 1999; Estoup et al. 2002; Perez-Losada et al. 2002).

Employing these methods we (1) investigate a possible genetic separation between *A. aranciacus* populations of the Atlantic and the Mediterranean basin and between populations in the eastern and the western Mediterranean; (2) test existing populations for isolation-by-distance versus panmixia within and/or among the basins; and (3) determine the degree of correlation between genetic differentiation patterns estimated from mtDNA sequence data and patterns resulting from microsatellite loci.

Materials and methods

Sampling and molecular methods

We sampled a total of 254 individuals from 9 locations within the Mediterranean Sea and the east Atlantic as shown in Fig. 1. Specimens were obtained by scuba diving and from commercial trawl and gill net operations within the years 2002 to 2006. Samples were preserved in 96% ethanol or in 80% ethanol buffered with DMSO until processed. We extracted DNA of approximately 30 mg of arm tip tissue or tube feet using a DNeasy Tissue Kit® (QIAGEN) following the manufacturer's instructions for extraction of animal tissue for a final volume of 400 µl. Extracted DNA was stored at −20°C.

We amplified fragments of the mitochondrial DNA (mtDNA) for 15–20 specimens per location according to Table 1 using the forward primer E12Sa and the reverse primer E16Sb as described in Smith et al. (1993). These primers amplify a fragment of approximately 1,200 base pairs (bp) in *A. aranciacus* and contain part of the 16S ribosomal RNA region, the entire non-coding control region (CR), the tRNA-Thr/Glu regions and part of the 12S ribosomal RNA region. DNA amplifications were performed in 30 µl-volume reactions with 1.67 U *Taq* DNA *Polymerase*, 3 µl 10× PCR reaction buffer, 0.4 mM dNTPs, 0.2 µM of each primer, 1 mM $MgCl_2$ and 6 µl of DNA. The PCR protocol consisted of an initial denaturation step at 95°C for 3 min, 40 amplification cycles (95°C for 30 s, 48°C for 30 s and 72°C for 1 min) and a final elongation step at 72°C for 10 min performed in a Whatman Biometra T1 Thermocycler. We purified the PCR products with the QIAquick® PCR Purification Kit (Qiagen) or NucleoSpin® Extract II (Macherey-Nagel AG, Oensingen, Switzerland), following the supplier's instructions. Forward and reverse sequencing using E12Sa and E16Sb were carried out separately using BigDye® Terminator (PE-Applied Biosystems) chemistry. The cycle-sequencing protocol consisted of an initial step at 96°C for 3 min and 24 sequencing cycles (96°C for 15 s, 50°C for 10 s and 60°C for 3 min). Cycle sequencing products were purified with a DyeEx™ 2.0 Spin Kit (Qiagen) or a NucleoSeq® (Macherey-Nagel) Purification Kit and sequenced on an ABI 3730 DNA Analyzer. Sequences were edited and aligned using the software SEQUENCHER™ 3.0 (Gene Codes Corporation) and adjusted by eye.

We analyzed nuclear DNA variation for all samples at four polymorphic microsatellite loci (Aaran06, Aaran09, Aaran2/05 and Aaran2/25) as previously characterized for *A. aranciacus* by Zulliger et al. (2008). DNA amplifications, fragment analyses and scoring were performed as described by Zulliger et al. (2008).

Genetic diversity within populations

MtDNA haplotypic diversity h (Nei 1987) and nucleotide diversity π (Tajima 1983) were calculated using ARLEQUIN version 3.11 (Excoffier et al. 2005). Moreover, we constructed a haplotype network using the statistical parsimony procedure of Templeton et al. (1992) implemented in TCS version 1.21 (Clement et al. 2000) with gaps coded as

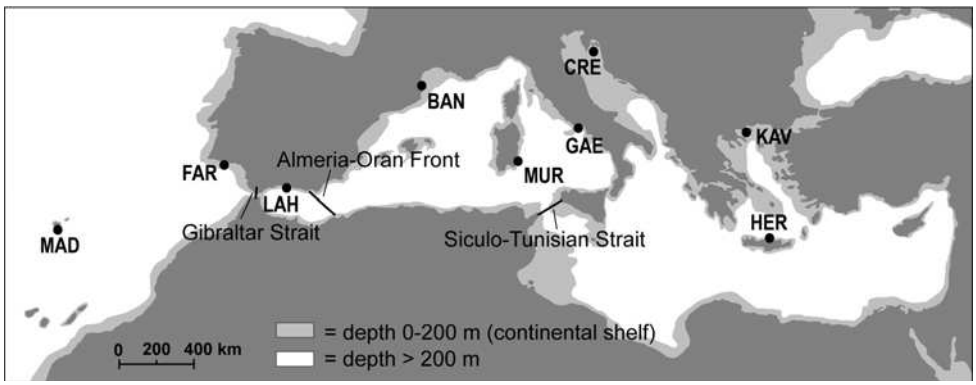

Fig. 1 Map showing the sampling sites of *A. aranciacus*. *MAD* Madeira, *FAR* Faro, *LAH* La Herradura, *BAN* Banyuls, *MUR* Muravera, *GAE* Gaeta, *CRE* Cres, *HER* Heraklion, *KAV* Kavala

Table 1 *A. aranciacus* intra-population genetic variability at the mitochondrial control region (CR) including flanking regions and four microsatellite loci for nine sampling locations

Location	MtDNA					Microsatellite loci																Mean R_s
	CR and flanking regions					*n*	Aaran06				Aaran09				Aaran2/05				Aaran2/25				
	n	*T*	*S*	*h*	π		N_a	H_O	H_E	R_s	N_a	H_O	H_E	R_s	N_a	H_O	H_E	R_s	N_a	H_O	H_E	R_s	
MAD	19	12	11	0.8363 (0.0199)	0.0029 (0.0004)	21	9	0.810	0.832	8.949	3	0.429	0.547	3.000	6	0.667	0.667	5.952	18	0.700* $r = 0.112$	0.923	18.000	8.975
FAR	18	15	12	0.9804 (0.0057)	0.0062 (0.0008)	30	11	0.759	0.798	10.149	5	0.724	0.725	4.900	9	0.625	0.710	7.943	22	0.704*** $r = 0.121$	0.941	18.860	10.463
Total Atlantic	*37*	*27*	*23*	*0.9535 (0.0044)*	*0.0050 (0.0005)*	*51*	*12*	*0.780*	*0.810*	*9.549*	*5*	*0.600*	*0.671*	*3.950*	*9*	*0.640*	*0.695*	*6.948*	*30*	*0.702*** $r = 0.127$*	*0.951*	*18.430*	*9.719*
LAH	15	13	7	0.9714 (0.0100	0.0069 (0.0010)	22	12	0.864	0.863	11.539	4	0.727	0.718	4.000	11	0.864	0.848	10.545	17	0.727*** $r = 0.105$	0.943	16.597	10.670
BAN						23	9	0.652°	0.788	8.709	7	0.696	0.620	6.580	10	0.739	0.864	9.810	19	0.619** $r = 0.162$	0.945	18.567	10.917
MUR	20	20	13	1.0000 (0.0035)	0.0054 (0.0007)	49	14	0.886	0.837	11.627	6	0.818***	0.648	5.115	14	0.773	0.815	10.567	25	0.757* $r = 0.096$	0.95	19.904	11.803
GAE	20	19	12	0.9947 (0.0040)	0.0072 (0.0009)	28	12	0.893	0.860	10.679	6	0.643	0.604	5.330	11	0.893	0.823	9.773	25	0.857°	0.96	21.138	11.730
Total W. Med.	*55*	*44*	*38*	*0.9892 (0.0008)*	*0.0064 (0.0005)*	*122*	*15*	*0.838*	*0.838*	*10.639*	*7*	*0.735****	*0.646*	*5.256*	*17*	*0.812*	*0.834*	*10.174*	*36*	*0.750*** $r = 0.117$*	*0.954*	*19.052*	*11.280*
CRE	20	18	14	0.9895 (0.0043)	0.0065 (0.0008)	21	10	0.750	0.837	10.000	6	0.619°	0.714	5.950	8	0.714	0.816	7.951	24	0.900°	0.968	24.000	11.975
HER	20	19	17	0.9947 (0.0040)	0.0078 (0.0009)	30	12	0.933	0.881	11.197	6	0.467	0.652	5.226	12	0.833°	0.904	11.482	24	0.900	0.958	20.311	12.054
KAV	19	18	14	0.9942 (0.0044)	0.0067 (0.0008)	30	12	0.733	0.847	10.441	5	0.667	0.627	4.333	11	0.897	0.866	9.493	28	0.767*** $r = 0.098$	0.968	23.131	11.850
Total E. Med.	*59*	*51*	*47*	*0.9930 (0.0007)*	*0.0069 (0.0005)*	*81*	*15*	*0.813*	*0.854*	*10.546*	*8*	*0.580*	*0.654*	*5.170*	*12*	*0.825*	*0.869*	*9.642*	*37*	*0.850*** $r = 0.057$*	*0.966*	*22.481*	*11.960*
All pops	*151*	*114*	*100*	*0.9910 (0.0002)*	*0.0064 (0.0003)*	*254*	*17*	*0.818*	*0.838*	*10.598*	*9*	*0.657*	*0.657*	*5.246*	*18*	*0.781*	*0.826*	*9.841*	*46*	*0.774** $r = 0.095$*	*0.960*	*21.917*	*11.901*

Indicated are the number of individuals processed (*n*) for mitochondrial and nuclear markers, as well as the number of total haplotypes (*T*), the number of location-specific haplotypes found within each sampling location (*S*) and haplotype (*h*) and nucleotide (π) diversity. Standard errors are in parenthesis. For microsatellite loci the number of alleles (N_a), expected (H_E) and observed heterozygosity (H_O) and the allelic richness (R_s) are shown. Allelic richness is adjusted for a minimum sample size of 20 diploid individuals. Estimated null allele frequencies (*r;* van Oosterhout) are shown for populations with significant lower H_O from HWE after false discovery rate (FDR) correction

Italicized numbers indicate the total of a region and the total of over all samples

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ° = values that become non-significant after applying FDR correction

5th character state and a 95% connection limit. To test for possible nucleotide saturation, we obtained saturation plots using the program DAMBE (Data Analysis in Molecular Biology and Evolution; Xia and Xie 2001).

Microsatellite allelic diversity (N_a), allele frequencies and allelic richness corrected for differences in samples size (R_s; ElMousadik and Petit 1996) were determined per locus and per sampling location using the software FSTAT version 2.9.3.2 (Goudet 1995). Further, we calculated observed (H_O) and expected heterozygosity (H_E) in ARLEQUIN and tested for significant deviations from Hardy–Weinberg equilibrium (HWE) as described in Guo and Thompson (1992) using 1,000,000 steps in the Markov chain and 50,000 dememorization steps. Tests for linkage disequilibrium were also performed in ARLEQUIN using a likelihood-ratio test (Slatkin and Excoffier 1996) and 16,000 random permutated samples. To detect microsatellite scoring errors, large allele dropout, occurrence of null alleles and estimates of null allele frequency, we used the software MICRO-CHECKER version 2.2.1 (Van Oosterhout et al. 2004).

As the haplotype diversity (h) and the allelic richness (R_s) were noticeably lower in Madeira (MAD) than in the other locations, we performed a one-sample t test using the statistics software SPSS 14.0 to test for statistical significance of this difference.

Bayesian clustering

STRUCTURE version 2.0 (Pritchard et al. 2000) was used to infer population genetic structure testing the consistency with microsatellite genetic information. This Bayesian clustering method takes a sample of genotypes and uses the assumption of HWE and linkage equilibrium within subpopulations to find the number of populations (K) that fits the data best and the individual assignments that minimize Hardy–Weinberg and linkage disequilibrium in those populations. 10 replicates of this analysis were performed with K ranging from 1 to 12 for 1,000,000 generations (burn-in 100,000) and assuming an admixture model.

Genetic differentiation among populations

Population pairwise F_{ST} estimates (Weir and Cockerham 1984; Michalakis and Excoffier 1996) were calculated using ARLEQUIN for mtDNA and microsatellite data applying 16,000 permutations and Kimura 2-parameter corrected distances for mtDNA. Estimates of F_{ST} based on microsatellite data were also carried out without locus Aaran2/25, as null alleles are likely to be present at this locus (see below). To test whether F_{ST} using all four loci and F_{ST} without locus Aaran2/25 differed significantly, a paired sample t test was performed with the statistics software SPSS. This test was not significant ($P = 0.648$, correlation = 0.948), and thus, all further calculations based on F_{ST} were carried out using all four loci. For microsatellite data we also calculated the standardized genetic differentiation measure as proposed by Hedrick (2005) which accounts for the level of genetic variation. To calculate this measure, all alleles were recoded as being population specific using the program RecodeData (Meirmans 2006).

We examined the partitioning of the total variance between various groups of samples in ARLEQUIN by performing hierarchical analysis of molecular variance (AMOVA; Excoffier et al. 1992). Based on allelic frequencies for microsatellite loci and applying Kimura 2-parameter corrected distances for mtDNA, multilevel AMOVAs were performed to examine the proportion of genetic variance among the Atlantic and Mediterranean basins and within the Mediterranean among the west and the east basins separated by the Siculo-Tunisian strait (see Fig. 1). As the sampling location La Herradura (LAH) is located in the Alboran Sea, west of the Almería-Oran front, AMOVAs were carried out both with LAH belonging to the Atlantic and with LAH belonging to the west Mediterranean (16,000 permutations).

To estimate the effects of isolation-by-distance, we calculated the correlation between pairwise genetic differentiation (F_{ST}) and geographic distance using the software IBD version 1.52 (Bohonak 2002). IBD uses a Mantel-test to find relationships between genetic and geographic distance matrices. As proposed by Rousset (1997) for a two-dimensional dispersal, we compared $F_{ST}/(1 - F_{ST})$ with the logarithm of the geographic distance. Isolation-by-distance was tested for distances measured as the direct sea path between sampling locations (10,000 randomizations). As pairwise genetic differentiation (F_{ST}) of Madeira (MAD) to most other locations was significant, we performed a second analysis without this location to test whether the correlation was an artifact caused by the high genetic differentiation to MAD.

Correlation between mtDNA and microsatellite F_{ST}

We determined the correlation between the two matrices of population pairwise genetic differentiation (F_{ST}) resulting from mtDNA and microsatellite loci using a Mantel-test as applied in IBD (10,000 randomizations).

When multiple tests were performed, we corrected the level of significance according to the number of tests in a given set applying the control of false discovery rate (FDR) method (Benjamini and Hochberg 1995) as suggested by Narum (2006).

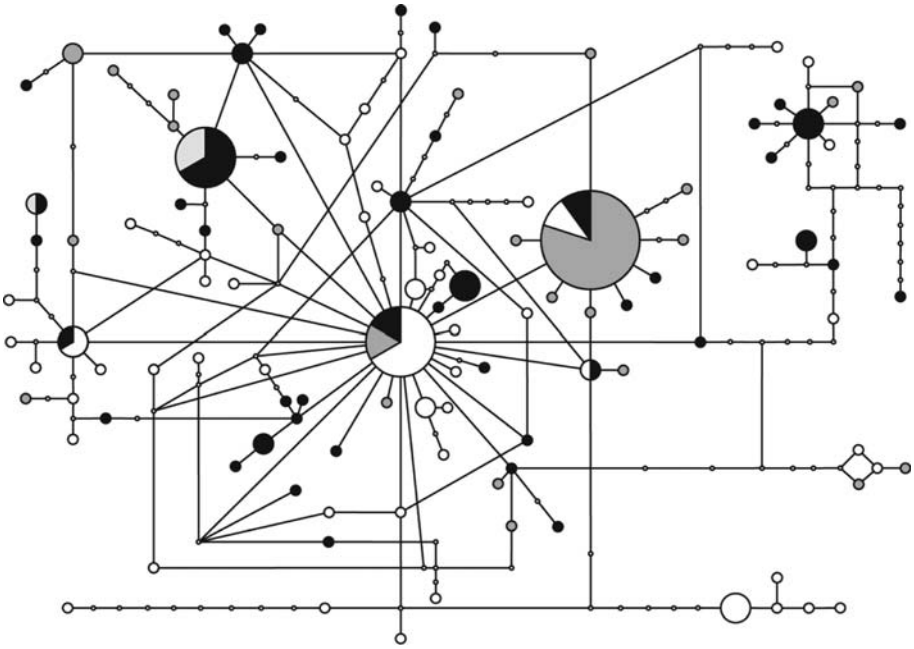

Fig. 2 Parsimony network showing relationships among all *Astropecten aranciacus* mtDNA haplotypes. Haplotypes are colored according to the region: Atlantic (*gray*), western Mediterranean (*black*), and eastern Mediterranean (*white*). *Circle* size is proportional to the number of samples with the same haplotype and size of pie slices to the number of samples from the same region. *Each line* in the network represents a single mutational change. *Small dots* indicate intermediate haplotypes not observed in the sample but necessary to link all observed haplotypes to the network

Results

Genetic diversity within populations

We successfully amplified a 1,017 bp fragment of mtDNA containing the control region in 151 individuals across eight Atlantic and Mediterranean sampling locations (see Fig. 1, Table 1). All sequences were deposited in GenBank under accession numbers EU450469–EU450582. The sample from Banyuls (BAN) was not included in the mtDNA analyses, because it was not possible to amplify the desired fragment of enough individuals from this location.

A total of 114 unique haplotypes were identified of which the two most common haplotypes (no. 1 and 72) were found in all three regions (see Electronic supplementary material for absolute haplotype frequencies). We observed 96 polymorphic sites (9.4% variable sites), of which 12 contained gaps, and the number of nucleotide substitutions between any pair of sequences ranged from 1 to 25. Mean haplotype diversity (h) equaled 0.99 with the highest h observed in the sample from Muravera (MUR; $h = 1.00$) and the lowest h in Madeira (MAD; $h = 0.84$). A one-sample t test revealed a significantly lower h in MAD compared to other populations (mean difference = 13.385; $P < 0.001$). Mean nucleotide diversity (π) was 0.0064, and no evidence of nucleotide saturation was present in the saturation plot produced by DAMBE (results not shown).

One minimum spanning haplotype network with numerous ambiguous connections was obtained using TCS (Fig. 2). A central haplotype could be identified in all three regions, and the most frequent haplotypes were generally closely linked to each other.

For the majority of the individuals, all four microsatellite loci amplified successfully and were scored unambiguously (Table 1). At loci Aaran06 and Aaran2/05 one individual each did not amplify, and at locus Aaran2/25 amplifications for 13 individuals from five different sampling locations were not successful. All loci were polymorphic at each sampling location, and allelic diversity (N_a) within populations ranged from three alleles in Aaran09 to 28 alleles in Aaran2/25 (Table 1). There was no evidence of scoring errors due to stutter or large allele dropout. Observed (H_O) and expected heterozygosity (H_E) ranged from 0.429 to 0.933 and from 0.547 to 0.968, respectively. Except for locus Aaran2/25, none of the loci showed evidence of the presence of null alleles. Deviations from Hardy–Weinberg equilibrium (HWE) were detected at locus Aaran2/25 after correcting for multiple testing (initial $\alpha = 0.05$, $k = 4$) for the samples Madeira (MAD), Faro (FAR), La Herradura (LAH), Banyuls (BAN), Muravera (MUR), Kavala (KAV) and for all samples combined. The presence of one or more null alleles at locus Aaran2/25 was indicated by an excess of homozygotes over most size classes, detected by the program MICROCHECKER, and by the failure to amplify this locus in several specimens. In contrast, a significant excess of heterozygotes was detected at locus Aaran09 within the population MUR. Evidence of linkage disequilibrium between pairs of loci among populations was only significant at Gaeta (GAE) for loci Aaran06 and Aaran2/25 ($P = 0.005$), while over all populations no loci were significantly linked. Allelic richness adjusted for differences in sample size ranged from 3.000 in MAD at Aran09 to 23.131 in KAV at Aaran2/25 (Table 1). Overall allelic richness as an average of the four loci was the highest in

Heraklion (HER) with 12.054 and the lowest in MAD with 8.975 (Table 1). A one-sample *t* test showed a significantly lower allelic richness in MAD compared to the other locations (mean difference = 2.180; $P < 0.001$). Over all microsatellite loci, 18.7% of the alleles were population-specific, whereas the lowest percentage of population specific alleles equaled 15.2% at locus Aaran2/25. The number of population specific alleles ranged from two at Aaran09 to seven at Aaran2/25 and amounted to a total of 17 alleles over all loci.

Bayesian clustering

Structure analysis of microsatellite data failed to distinguish among the nine locations, as all ten runs exhibited the best $-\log \mathrm{PR}(X|K)$ estimates for $K = 1$ ($\ln = -4273.5$; Fig. 3). We repeated this analysis without locus Aaran2/25, since this locus demonstrated deviation from HWE, however, the best $-\log \mathrm{PR}(X|K)$ estimates remained for $K = 1$ (5 runs; $\ln = -2781.7$; results not shown).

Genetic differentiation among populations

MtDNA pairwise F_{ST} ranged from 0 to 0.19 and provided evidence of a genetic subdivision between MAD and all other locations (Table 2). Microsatellite loci revealed a similar genetic differentiation pattern, but in addition to MAD, F_{ST} were also significant between FAR and several Mediterranean populations. F_{ST} in microsatellites ranged from zero to 0.0312 and were on average 5.9-fold lower than in mtDNA (Table 2). Nevertheless, a Mantel-test showed significant correlation between mitochondrial and microsatellite F_{ST} ($r = 0.84$; $P = 0.0127$). Standardized genetic differentiation in microsatellite data ranged from 0 to 0.15 and was on average 1.5-fold lower than in mtDNA.

Analyses of molecular variance (AMOVA) revealed high levels of variation within populations for both mtDNA and microsatellites ranging from 94.15 to 97.21% and from 98.75 to 99.69%, respectively (Table 3). In mtDNA, variation among populations was the highest when comparing the west Mediterranean (without LAH) versus the east Mediterranean populations (3.94%). However, none of the groupings showed significant among group variation in mtDNA after correcting for multiple testing. In microsatellites, the highest percentage of among group variation was achieved when clustering the Atlantic versus the Mediterranean populations (0.8%; Table 3). Control of false discovery rate (FDR) left all groupings significant, except for one (WMed without LAH vs. EMed).

Isolation-by-distance analysis by the nearest sea path resulted in a significant correlation of genetic differentiation and geographic distance in both mtDNA ($P = 0.0043$; Fig. 4a) and microsatellite loci ($P = 0.0023$; Fig. 4b). Even when omitting the sample MAD from the analysis, this correlation remained significant in both markers ($P_{mtDNA} = 0.0261$; $P_{msat} = 0.0038$). As shown in Fig. 4b, pairs with MAD (empty dots) clearly have higher F_{ST} than could be expected by the geographic distance, as well as one outlier pair with FAR and BAN (filled dot).

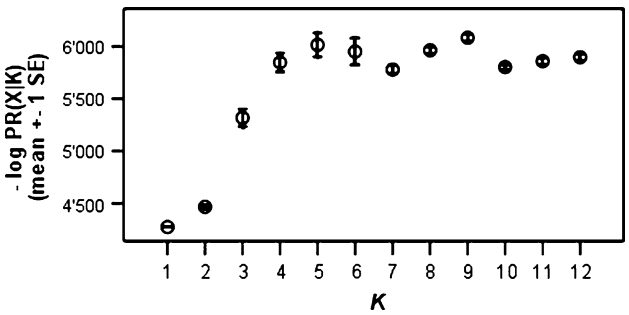

Fig. 3 Results of the structure analysis using microsatellite data indicating the average $-\log \mathrm{PR}(X|K)$ estimate with standard error bars for each number of populations (K)

Discussion

In this study we used both mitochondrial and microsatellite markers to investigate the genetic structure in a high dispersal echinoderm in the Atlanto-Mediterranean region. Our results showed that the mitochondrial control region is highly variable and the four microsatellite loci Aaran06, Aaran09, Aaran2/05 and Aaran2/25 are also highly polymorphic in *A. aranciacus*. The comparison of these two markers allows us to determine the population structure in this sea star in a comprehensive manner, as our mitochondrial and nuclear data in general exhibit a congruent pattern of genetic differentiation.

Within population variability

While nucleotide diversity was low in mtDNA sequence data, haplotype diversity was high, indicating a high degree of polymorphism in the mitochondrial control region. Other studies on echinoderms have obtained similar results for sequences of the mitochondrial cytochrome c oxidase subregion I (COI) (e.g., McCartney et al. 2000; Uthicke and Benzie 2003; Duran et al. 2004a). In marine invertebrates with large population sizes numerous haplotypes can be retained during periods of population growth or expansion (Watterson 1984). A rapid population expansion could therefore lead to high haplotype and low nucleotide diversity as new mutations are retained (Avise et al. 1984; Watterson 1984). In the present case, the results of the parsimony network analysis for *A. aranciacus* seem to support this hypothesis. The network showed a star-shaped

Table 2 Population pairwise genetic differentiation F_{ST} between sampling locations of *A. aranciacus* based on mtDNA sequence data and F_{ST} and standardized genetic differentiation in four microsatellite loci

	Atlantic		West Mediterranean				East Mediterranean		
	MAD	FAR	LAH	BAN	MUR	GAE	CRE	HER	KAV
mtDNA									
MAD	–	**0.0000**	**0.0000**		**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**
FAR	0.1796	–						0.0488	0.0396
LAH	0.1666	−0.0253	–						
BAN				–					
MUR	0.1711	0.0039	−0.0045		–				0.0397
GAE	0.1884	−0.0014	−0.0151		−0.0147	–		0.0489	0.0381
CRE	0.1378	−0.0043	0.0011		0.0020	0.0105	–		
HER	0.1630	0.0428	0.0257		0.0337	0.0437	0.0026	–	
KAV	0.1663	0.0463	0.0351		0.0419	0.0510	0.0007	−0.0317	–
Microsatellite loci									
MAD	–	**0.0046**	**0.0040**	**0.0039**	**0.0114**	**0.0160**	**0.0315**	**0.0013**	**0.0029**
FAR	0.0254	–		**0.0059**		**0.0273**		**0.0030**	**0.0083**
	0.1079								
LAH	0.0299	−0.0010	–						
	0.1443	*0.0000*							
BAN	0.0312	0.0231	0.0052	–					
	0.1361	*0.1129*	*0.0296*						
MUR	0.0165	0.0010	−0.0027	0.0016	–				
	0.0716	*0.0048*	*0.0000*	*0.0080*					
GAE	0.0178	0.0125	0.0068	0.0021	−0.0039	–			
	0.0809	*0.0635*	*0.0400*	*0.0111*	*0.0000*				
CRE	0.0197	0.0073	0.0032	0.0094	0.0024	−0.0004	–		
	0.0914	*0.0383*	*0.0198*	*0.0514*	*0.0129*	*0.0000*			
HER	0.0305	0.0213	0.0055	0.0051	0.0061	0.0034	0.0017	–	
	0.1529	*0.1204*	*0.0366*	*0.0302*	*0.0366*	*0.0207*	*0.0110*		
KAV	0.0251	0.0167	−0.0003	0.0003	0.0020	−0.0009	0.0004	−0.0069	–
	0.1177	*0.0879*	*0.0000*	*0.0016*	*0.0108*	*0.0000*	*0.0024*	*0.0000*	

Below diagonal: multilocus F_{ST} (Weir and Cockerham 1984) and standardized genetic differentiation (Hedrick 2005) in italic (for microsatellite loci only); above diagonal: P values < 0.05. Significance is indicated in bold after false discovery rate correction

pattern with a central haplotype and various alternative connections. The star-shape possibly indicates a common ancestral haplotype (Templeton et al. 1995), supported by the occurrence of a central haplotype in all three basins (Atlantic, western and eastern Mediterranean).

Microsatellite loci were also highly polymorphic and showed a high mean allelic richness per population ($R_s = 21.9$), but only few alleles were population specific. The lowest percentage of population specific alleles was found at locus Aaran2/25 (15.2%). Together with the high allelic richness at this locus, this could be an indication of size homoplasy. According to Estoup et al. (2002), this would not necessarily be a significant problem for many types of population genetic analyses. However, highly polymorphic loci due to high mutation rates can lead to lower estimates of F_{ST} (Slatkin 1995), and high levels of heterozygosity may reduce the relationship between statistical and biological significance (Hedrick 1999). As F_{ST} estimates with and without locus Aaran2/25 showed no significant difference, we can assume that neither homoplasy nor the presence of null alleles skew the results of this study. Moreover, multiallelic F_{ST} is based on a weighted mean of the contribution to the overall variance of each allele considered separately (Weir and Cockerham 1984). It is therefore not likely to be biased by an invisible allele, which is expected to be randomly associated with size scored alleles (Launey et al. 2002).

For some groups of invertebrates the proportion of microsatellite loci without null alleles is often low (McGoldrick et al. 2000; Launey et al. 2002; Peijnenburg et al. 2006; Costantini et al. 2007). Possibly, this could be due to a less effective DNA repair mechanism in the nuclear DNA of some invertebrates, especially in echinoderms. Primers for amplification of microsatellite loci have been developed for several other echinoderms, such as *Acanthaster plancii* (Yasuda et al. 2006), *Amphipholis squamata* and

Table 3 Analysis of molecular variance (AMOVA) based on pairwise distances in *A. aranciacus* for different groupings of Atlantic (A) versus Mediterranean (M) and within the Mediterranean for eastern (EM) versus western (WM) samples

Grouping the sample LAH to a basin other than the western Mediterranean is indicated by +, removing it by -. The table includes the degrees of freedom (*df*) for both mitochondrial DNA (mtDNA) and microsatellite loci (msats) and the F-statistics. Only *P* values >0.05 are listed, and significance after false discovery rate correction is indicated in bold

–, Non significant ($P \geq 0.05$)

Grouping	*df*		Percentage of variation		Fixation indices		*P*	
	mtDNA	msats	mtDNA	msats	mtDNA	msats	mtDNA	msats
A+ vs. M								
Among groups	1	1	1.39	0.52	$F_{CT} = 0.013$	0.005	–	**0.035**
Within groups	6	7	3.94	0.500	$F_{SC} = 0.039$	0.005	**0.005**	0.035
Within populations	143	487	94.68	98.98	$F_{ST} = 0.053$	0.010	**0.000**	**0.002**
A vs. M								
Among groups	1	1	2.11	0.80	$F_{CT} = 0.021$	0.008	–	**0.029**
Within groups	6	7	3.73	0.45	$F_{SC} = 0.038$	0.005	**0.000**	0.049
Within populations	143	487	94.15	98.75	$F_{ST} = 0.058$	0.013	**0.000**	**0.002**
A+ vs. WM vs. EM								
Among groups	2	2	2.47	0.48	$F_{CT} = 0.024$	0.005	0.043	**0.014**
Within groups	5	6	2.79	0.39	$F_{SC} = 0.028$	0.004	**0.029**	–
Within populations	143	487	94.73	99.13	$F_{ST} = 0.052$	0.009	**0.000**	**0.002**
A vs. WM vs. EM								
Among groups	2	2	3.24	0.60	$F_{CT} = 0.032$	0.006	0.014	**0.007**
Within groups	5	6	2.22	0.32	$F_{SC} = 0.022$	0.003	**0.002**	–
Within populations	143	487	94.54	99.08	$F_{ST} = 0.054$	0.009	**0.000**	**0.002**
WM vs. EM								
Among groups	1	1	3.79	0.37	$F_{CT} = 0.037$	0.004	–	**0.028**
Within groups	4	5	−1.01	−0.06	$F_{SC} = -0.010$	−0.001	–	–
Within populations	108	389	97.21	99.69	$F_{ST} = 0.027$	0.003	–	–
WM− vs. EM								
Among groups	1	1	3.94	0.47	$F_{CT} = 0.039$	0.005	–	–
Within groups	3	4	−0.96	−0.13	$F_{SC} = -0.010$	−0.001	–	–
Within populations	94	346	97.02	99.66	$F_{ST} = 0.029$	0.003	–	–

Echinocardium cordatum (Chenuil et al. 2003), *Strongylocentrotus spp.* (Addison and Hart 2002), *Parastichopus californicus* (Nelson et al. 2002), *Apostichopus japonicus* (Zhan et al. 2007), *Tripneustes gratilla* (Carlon and Lippe 2007) and *Evechinus chloroticus* (Perrin and Roy 2000).

Haplotype and allelic diversity are significantly lower in the sample from Madeira (MAD) than in all other samples, which could reflect either a founder event during the colonization of Atlantic islands or a recent bottleneck. The population from MAD was most likely not closely linked to the coastal populations in the past, as the island of Madeira emerged volcanically at the most 5 Mio years ago (Geldmacher and Hoernle 2000). Lower allelic richness has also been found in populations of *Crambe crambe* in the Canaries and Madeira archipelagos compared to Mediterranean populations (Duran et al. 2004b). Along with a reduction of alleles, an excess of heterozygosity would be a sign of a recent bottleneck, as allele number usually decreases faster than heterozygosity (Cornuet and Luikart 1996). We tested for a recent reduction in population size applying the software BOTTLENECK version 1.2.02 (Cornuet and Luikart 1996), but could not detect a bottleneck for MAD. Nevertheless, this possibility can not be completely ruled out, and additional microsatellite loci would be necessary to gain more clarity. Furthermore, genetic patterns are similar both in founder events and recent bottlenecks, and thus both possibilities still remain. Using mtDNA we performed a mismatch distribution analysis and Tajima's D test of selective neutrality (Tajima 1989) in ARLEQUIN to explore the demographic past of *A. aranciacus* in Madeira. This test showed that the population in MAD has undergone a recent population expansion, as it did not differ significantly from the sudden expansion model by Rogers and Harpending (1992).

Spatial structure

While Bayesian clustering (STRUCTURE) of microsatellite data failed to detect any genetic structure in *A. aranciacus*, pairwise genetic differentiation (F_{ST}) was significant mainly between MAD and all the other samples. These results are not contradictory given the low estimates of F_{ST} in microsatellites and that STRUCTURE tries to assign individuals to a population without any initial information about the true sampling location. Mitochondrial and nuclear data in general showed congruent genetic differentiation

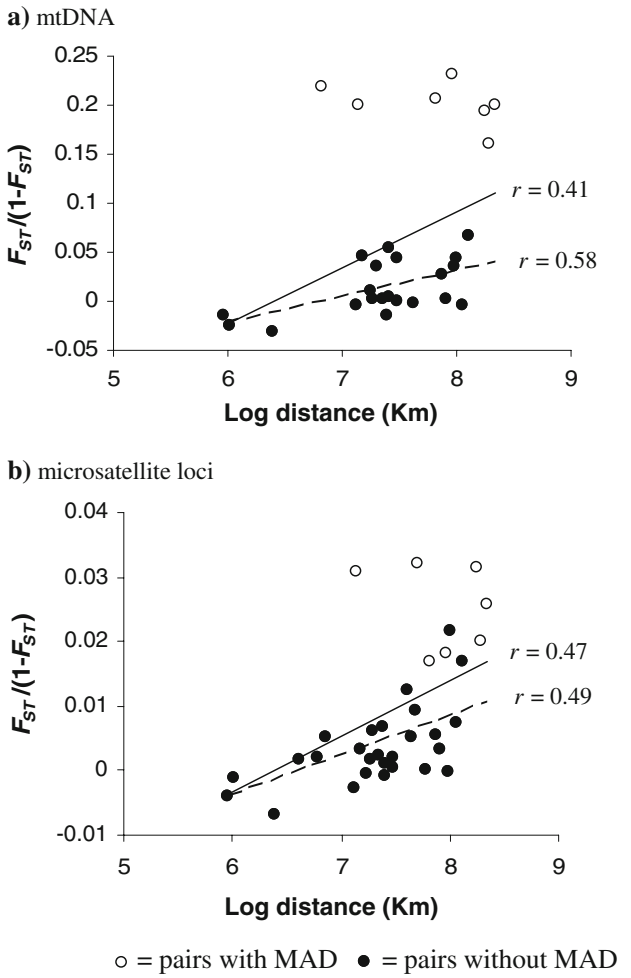

Fig. 4 Genetic differentiation [computed as $F_{ST}/(1 - F_{ST})$] versus the logarithm of the geographic distance in km in *A. aranciacus* based on **a** mtDNA sequence data and **b** microsatellite data. *Full lines* represent the regression line over all pairwise F_{ST}, *dotted lines* represent the regression line omitting the location MAD

patterns. The significant genetic differentiation between MAD and the other populations revealed by mitochondrial and microsatellite data can be explained by the remoteness of MAD to the other sites and possibly also by currents which are unfavorable to movement from the shelf to Madeira and vice versa. The Portugal current, which flows along the east Atlantic coast from northern Portugal southwards to northern Africa, is a possible marine barrier to gene-flow from the Mediterranean and east Atlantic coast to the island of Madeira. Moreover, the Mediterranean outflow tends to stratify in the Atlantic Ocean at a depth of 600–1,400 m due to its greater density (Mougenot and Vanney 1982) and might impede larval dispersal to Madeira. On the other hand, larvae dispersing from Madeira are prone to be directed southward along with the Canary current, restricting gene-flow to the east Atlantic coast and the Mediterranean.

Although highly correlated, F_{ST} (θ) were several times higher in mitochondrial than in nuclear markers. The average cytoplasmic/nuclear ratio (θ_C/θ_N) equaled 5.9. Similar results have been found in several other studies comparing mtDNA and microsatellite data (e.g., Shaw et al. 1999; Krafsur 2002; Diaz-Almela et al. 2004; Lemaire et al. 2005; Peijnenburg et al. 2006). As the effective mitochondrial population size (N_e) is expected to be four times smaller than the nuclear N_e, assuming both 1:1 sex ratios and diploidy (Birky et al. 1983), there is a higher potential for genetic drift in mtDNA, which leads to a higher θ. Ratios significantly higher than fourfold can be caused by sex bias in migration, reproduction or population size or by a difference in the mutation rate of the two markers (Turan et al. 1998; Shaw et al. 1999). The average θ_C/θ_N ratio in *A. aranciacus* is slightly higher than fourfold, for which the mentioned possibilities of sex bias or differences in mutation rates, combined or separately, could be partly responsible. A sex bias in migration though is an improbable factor, given the non-migratory behavior of *A. aranciacus*. The standardized measure of genetic differentiation for microsatellite data (Table 2) is almost comparable to F_{ST} in mtDNA and shows that low values of F_{ST} in microsatellite loci are mostly due to the high polymorphism in these markers.

Mitochondrial data failed to indicate any significant among group variation for any of the groups formed for the analysis of molecular variance (AMOVA) after correcting for multiple testing. In microsatellite loci, however, significant among group variation was found for all groupings except for one, indicating some differentiation between the basins. Since the Atlantic versus Mediterranean differentiation might be skewed by the sample from Madeira (MAD), further sampling along the east Atlantic coast, e.g., North Africa, is needed in order to clarify whether there is a true genetic differentiation between these two basins, or if the pattern observed here is limited to offshore islands. As for the Mediterranean, our data suggest significant among group variation between populations of *A. aranciacus* in the west and the east Mediterranean basins. A similar differentiation between eastern (Adriatic) and western Mediterranean populations has been found in other high dispersal marine species, such as the chaetognath *Sagitta setosa* (Peijnenburg et al. 2006) and the bivalve C*erastoderma glaucum* (Mariani et al. 2002). Although AMOVA indicate some genetic differentiation between basins in populations of *A. aranciacus*, this variation could be connected to the clear isolation-by-distance pattern found in *A. aranciacus*, as grouping populations by basin includes a strong geographical component. This hypothesis deserves further consideration, as in most cases significance of among group variation did not depend on whether LAH was assigned to the Mediterranean or to the Atlantic group.

Analyses of isolation-by-distance (IBD) were significant in both mtDNA and microsatellite data regardless of whether MAD was included in the analysis or not. We can therefore rule out a possible artifact caused by MAD, which showed a significant genetic differentiation (F_{ST}) to most other populations. However, pairwise F_{ST} comparisons between MAD and the other study sites are much higher than expected from the regression line resulting from IBD analyses (see Fig. 4). In fact, F_{ST} values between MAD and the other populations are about five times higher in mtDNA and twice as high in microsatellites. Besides the large geographic distance, other isolation mechanisms must therefore be acting on the population of MAD, such as the unfavorable current regime discussed above. Why the pair FAR-BAN also exhibits such a high F_{ST} in microsatellites still needs to be further investigated by either obtaining comparable mtDNA data from BAN or by sampling other populations of the west coast of the Mediterranean.

Isolation-by-distance patterns have been detected in other Atlanto-Mediterranean invertebrates (Zane et al. 2000; Launey et al. 2002; Diaz-Almela et al. 2004) but not yet in high dispersal echinoderms. In contrast to our results, genetic investigations on the high dispersal sea urchin *Paracentrotus lividus* (Duran et al. 2004a) suggested panmixia within the Mediterranean and within the east Atlantic basin. This discrepancy is most likely due to the sampling scheme, as the presented study included populations in the eastern Mediterranean while the sampling by Duran et al. (2004a) was limited to the west coast of the western Mediterranean.

While mitochondrial and nuclear datasets reveal similar levels of significant genetic differentiation between samples, and both support an isolation-by-distance pattern, mtDNA showed higher population differentiation (F_{ST}) than microsatellite loci. However, when accounting for the high polymorphism in nuclear markers using the standardized genetic differentiation measure, this difference was less pronounced. On the other hand, microsatellite loci were more sensitive in detecting genetic differentiation between groupings by basins (AMOVA).

Conclusions

Our results indicate that while genetic differentiation may be mostly absent in *A. aranciacus*, the dispersal of marine invertebrates with extended planktonic larval stages could be more restricted than is often assumed. In contrast to previous studies on echinoderms suggesting panmixia in the Atlantic and Mediterranean basin, our data revealed a pattern of isolation-by-distance in *A. aranciacus* over the sampled area. Microsatellite data further detected some differentiation of Atlantic versus Mediterranean and western versus eastern Mediterranean populations of *A. aranciacus*. Nevertheless, our data did not allow to identify specific marine barriers, such as the Strait of Gibraltar, the Almería-Oran front or the Siculo-Tunisia Strait, as isolation-by-distance might be sufficient to explain the majority of the genetic differences found here. Further sampling, particularly along the Atlantic coast, is necessary to gain more clarity on this matter. The present study highlights how the comparison of mitochondrial and microsatellite markers can provide a more complete picture of genetic differentiation, allowing for more comprehensive data analyses and interpretation.

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References

Addison JA, Hart MW (2002) Characterization of microsatellite loci in sea urchins (*Strongylocentrotus* spp.). Mol Ecol Notes 493–494. doi:10.1046/j.1471-8286.2002.00295.x

Aquadro CF, Greenberg BD (1983) Human mitochondrial-DNA variation and evolution—analysis of nucleotide-sequences from 7 individuals. Genetics 103:287–312

Avise JC, Neigel JE, Arnold J (1984) Demographic influences on mitochondrial-DNA lineage survivorship in animal populations. J Mol Evol 20:99–105. doi:10.1007/BF02257369

Baus E, Darrock DJ, Bruford MW (2005) Gene-flow patterns in Atlantic and Mediterranean populations of the Lusitanian sea star *Asterina gibbosa*. Mol Ecol 14:3373–3382. doi:10.1111/j.1365-294X.2005.02681.x

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate—a practical and powerful approach to multiple testing. J R Stat Soc B Methodol 57:289–300

Birky CW, Maruyama T, Fuerst P (1983) An approach to population and evolutionary genetic theory for genes in mitochondria and chloroplasts, and some results. Genetics 103:513–527

Bohonak AJ (2002) IBD (isolation by distance): a program for analyses of isolation by distance. J Hered 93:153–154. doi:10.1093/jhered/93.2.153

Borsa P, Blanquer A, Berrebi P (1997) Zoogéographie intraspécifique de la mer Méditerranée. Analyse des données génétiques populationnelles sur seize espèces atlanto-méditerranéennes (Poissons et Invertèbres). Vie Milieu 47:95–305

Burla H, Pabst B, Stahel W (1976) Environmental-conditions affecting occurrence of *Astropecten-Aranciacus* (Asteroidea, Echinodermata). Helgol Wiss Meeresunters 28:167–182. doi:10.1007/BF01610351

Burla H, Ribi G, Ferlin V, Pabst B (1972) Notes on ecology of *Astropecten-Aranciacus*. Mar Biol (Berl) 14:235

Calderon I, Giribet G, Turon X (2008) Two markers and one history: phylogeography of the edible common sea urchin *Paracentrotus*

lividus in the Lusitanian region. Mar Biol (Berl) 154:137–151. doi:10.1007/s00227-008-0908-0

Carlon DB, Lippe C (2007) Eleven new microsatellite markers for the tropical sea urchin *Tripneustes gratilla* and cross-amplification in *Tripneustes ventricosa*. Mol Ecol Notes 7:1002–1004. doi:10.1111/j.1471-8286.2007.01755.x

Chenuil A, Le Gac M, Thierry M (2003) Fast isolation of microsatellite loci of very diverse repeat motifs by library enrichment in echinoderm species, *Amphipholis squamata* and *Echinocardium cordatum*. Mol Ecol Notes 3:324–327. doi:10.1046/j.1471-8286.2003.00434.x

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. Mol Ecol 9:1657–1659. doi:10.1046/j.1365-294x.2000.01020.x

Colgan DJ, Byrne M, Rickard E, Castro LR (2005) Limited nucleotide divergence over large spatial scales in the asterinid sea star *Patiriella exigua*. Mar Biol (Berl) 146:263–270. doi:10.1007/s00227-004-1415-6

Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001–2014

Costantini F, Fauvelot C, Abbiati M (2007) Genetic structuring of the temperate gorgonian coral (*Corallium rubrum*) across the western Mediterranean Sea revealed by microsatellites and nuclear sequences. Mol Ecol 16:5168–5182

Diaz-Almela E, Boudry P, Launey S, Bonhomme F, Lapegue S (2004) Reduced female gene flow in the European flat oyster *Ostrea edulis*. J Hered 95:510–516. doi:10.1093/jhered/esh073

Duran S, Palacin C, Becerro MA, Turon X, Giribet G (2004a) Genetic diversity and population structure of the commercially harvested sea urchin *Paracentrotus lividus* (Echinodermata, Echinoidea). Mol Ecol 13:3317–3328. doi:10.1111/j.1365-294X.2004.02338.x

Duran S, Pascual M, Estoup A, Turon X (2004b) Strong population structure in the marine sponge *Crambe crambe* (Poecilosclerida) as revealed by microsatellite markers. Mol Ecol 13:511–522. doi:10.1046/j.1365-294X.2004.2080.x

ElMousadik A, Petit RJ (1996) High level of genetic differentiation for allelic richness among populations of the argan tree *Argania spinosa* (L) Skeels endemic to Morocco. Theor Appl Genet 92:832–839. doi:10.1007/BF00221895

Estoup A, Jarne P, Cornuet JM (2002) Homoplasy and mutation model at microsatellite loci and their consequences for population genetics analysis. Mol Ecol 11:1591–1604. doi:10.1046/j.1365-294X.2002.01576.x

Excoffier L, Laval G, Schneider S (2005) ARLEQUIN (version 3.0): an integrated software package for population genetic data analysis. Bioinformatics Online 1:47–50

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes—application to human mitochondrial-DNA restriction data. Genetics 131:479–491

Féral J-P, Poulin E, Derelle E, Gallardo S, Chmbon C (1995) Genetic differentiation of *Echinocardium chordatum* as revealed by allozymes and RNA sequencing. In: Emson R, Smith A, Campbell A (eds) Echinoderm research 1995. Balkema, Rotterdam, pp 41–42

Geldmacher J, Hoernle K (2000) The 72 Ma geochemical evolution of the Madeira hotspot (eastern North Atlantic): recycling of Paleozoic (≤500 Ma) oceanic lithosphere. Earth Planet Sci Lett 183:73–92. doi:10.1016/S0012-821X(00)00266-1

Gerard K, Roby C, Chevalier N, Thomassin B, Chenuil A, Feral JP (2008) Assessment of three mitochondrial loci variability for the crown-of-thorns starfish: a first insight into Acanthaster phylogeography. C R Biol 331:137–143. doi:10.1016/j.crvi.2007.11.005

Goudet J (1995) FSTAT (Version 1.2): a computer program to calculate F-statistics. J Hered 86:485–486

Guo SW, Thompson EA (1992) Performing the exact test of Hardy–Weinberg proportion for multiple alleles. Biometrics 48:361–372. doi:10.2307/2532296

Hansen MM, Mensberg KLD, Berg S (1999) Postglacial recolonization patterns and genetic relationships among whitefish (*Coregonus* sp.) populations in Denmark, inferred from mitochondrial DNA and microsatellite markers. Mol Ecol 8:239–252. doi:10.1046/j.1365-294X.1999.00557.x

Harley CDG, Pankey MS, Wares JP, Grosberg RK, Wonham MJ (2006) Color polymorphism and genetic structure in the sea star *Pisaster ochraceus*. Biol Bull 211:248–262. doi:10.2307/4134547

Harper FM, Addison JA, Hart MW (2007) Introgression versus immigration in hybridizing high-dispersal echinoderms. Evolution 61:2410–2418. doi:10.1111/j.1558-5646.2007.00200.x

Harper FM, Hart MW (2005) Gamete compatibility and sperm competition affect paternity and hybridization between sympatric Asterias sea stars. Biol Bull 209:113–126. doi:10.2307/3593129

Hedrick PW (1999) Perspective: highly variable loci and their interpretation in evolution and conservation. Evolution 53:313–318. doi:10.2307/2640768

Hedrick PW (2005) A standardized genetic differentiation measure. Evolution 59:1633–1638

Hörstadius S (1938) Über die Entwicklung von *Astropecten aranciacus* L. Pubbl Stn Zool Napoli 17:221–312

Hunt A (1993) Effects of contrasting patterns of larval dispersal on the Genetic connectedness of local-populations of 2 intertidal starfish, Patiriella-Calcar and P-Exigua. Mar Ecol Prog Ser 92:179–186. doi:10.3354/meps092179

Koehler R (1921) Echinodermes. Faune de France, Lechevalier, Paris, pp 1–210

Krafsur ES (2002) Population structure of the tsetse fly *Glossina pallidipes* estimated by allozyme, microsatellite and mitochondrial gene diversities. Insect Mol Biol 11:37–45. doi:10.1046/j.0962-1075.2001.00307.x

Launey S, Ledu C, Boudry P, Bonhomme F, Naciri-Graven Y (2002) Geographic structure in the European flat oyster (*Ostrea edulis* L.) as revealed by microsatellite polymorphism. J Hered 93:331–338. doi:10.1093/jhered/93.5.331

Lemaire C, Versini JJ, Bonhomme F (2005) Maintenance of genetic differentiation across a transition zone in the sea: discordance between nuclear and cytoplasmic markers. J Evol Biol 18:70–80. doi:10.1111/j.1420-9101.2004.00828.x

Mariani S, Ketmaier V, de Matthaeis E (2002) Genetic structuring and gene flow in *Cerastoderma glaucum* (Bivalvia : Cardiidae): evidence from allozyme variation at different geographic scales. Mar Biol (Berl) 140:687–697. doi:10.1007/s00227-001-0753-x

Matsuoka N, Asano H (2003) Genetic variation in northern Japanese populations of the starfish Asterina pectinifera. Zool Sci 20:985–988. doi:10.2108/zsj.20.985

McCartney MA, Keller G, Lessios HA (2000) Dispersal barriers in tropical oceans and speciation in Atlantic and eastern Pacific sea urchins of the genus *Echinometra*. Mol Ecol 9:1391–1400. doi:10.1046/j.1365-294x.2000.01022.x

McGoldrick DJ, Hedgecock D, English LJ, Baoprasertkul P, Ward RD (2000) The transmission of microsatellite alleles in Australian and North American stocks of the Pacific oyster (*Crassostrea gigas*): selection and null alleles. J Shellfish Res 19:779–788

Meirmans PG (2006) Using the AMOVA framework to estimate a standardized genetic differentiation measure. Evolution 60:2399–2402

Michalakis Y, Excoffier L (1996) A generic estimation of population subdivision using distances between alleles with special reference for microsatellite loci. Genetics 142:1061–1064

Mougenot D, Vanney J-R (1982) The Plio-Quarternary sediment drifts of the south Portuguese continental slope. Bull Inst Geologie Bassin Aquitaine 31:131–139

Narum SR (2006) Beyond Bonferroni: less conservative analyses for conservation genetics. Conserv Genet 7:783–787. doi:10.1007/s10592-005-9056-y

Nei M (1987) Molecular evolutionary genetics. Colombia University Press, New York

Nelson RJ, Cooper G, Garner T, Schnupf P (2002) Polymorphic markers for the sea cucumber *Parastichopus californicus*. Mol Ecol Notes 2:233–235. doi:10.1046/j.1471-8286.2002.00205.x

Pabst B (1986) Eigenschaften der Dislokation bei drei Seesternarten der Gattung *Astropecten*. Inaugural-Dissertation, Universität Zürich

Palumbi SR, Grabowsky G, Duda T, Geyer L, Tachino N (1997) Speciation and population genetic structure in tropical Pacific Sea urchins. Evolution 51:1506–1517. doi:10.2307/2411203

Palumbi SR, Wilson AC (1990) Mitochondrial-DNA diversity in the Sea-Urchins *Strongylocentrotus-Purpuratus* and *Strongylocentrotus-Droebachie*. Evolution 44:403–415. doi:10.2307/2409417

Parsons TJ, Muniec DS, Sullivan K, Woodyatt N, AllistonGreiner R, Wilson MR, Berry DL, Holland KA, Weedn VW, Gill P, Holland MM (1997) A high observed substitution rate in the human mitochondrial DNA control region. Nat Genet 15:363–368. doi:10.1038/ng0497-363

Patarnello T, Volckaert F, Castilho R (2007) Pillars of Hercules: is the Atlantic–Mediterranean transition a phylogeographical break? Mol Ecol 16:4426–4444. doi:10.1111/j.1365-294X.2007.03477.x

Peijnenburg K, Fauvelot C, Breeuwer AJ, Menken SBJ (2006) Spatial and temporal genetic structure of the planktonic *Sagitta setosa* (Chaetognatha) in European seas as revealed by mitochondrial and nuclear DNA markers. Mol Ecol 15:3319–3338. doi:10.1111/j.1365-294X.2006.03002.x

Perez-Losada M, Guerra A, Carvalho GR, Sanjuan A, Shaw PW (2002) Extensive population subdivision of the cuttlefish *Sepia officinalis* (Mollusca : Cephalopoda) around the Iberian Peninsula indicated by microsatellite DNA variation. Heredity 89:417–424. doi:10.1038/sj.hdy.6800160

Perez-Losada M, Nolte MJ, Crandall KA, Shaw PW (2007) Testing hypotheses of population structuring in the Northeast Atlantic ocean and Mediterranean sea using the common cuttlefish *Sepia officinalis*. Mol Ecol 16:2667–2679. doi:10.1111/j.1365-294X.2007.03333.x

Perrin C, Roy MS (2000) Rapid and efficient identification of microsatellite loci from the sea urchin, *Evechinus chloroticus*. Mol Ecol 9:2221–2223. doi:10.1046/j.1365-294X.2000.105335.x

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Quesada H, Zapata C, Alvarez G (1995) A multilocus Allozyme discontinuity in the Mussel *Mytilus-Galloprovincialis*—the interaction of ecological and life-history factors. Mar Ecol Prog Ser 116:99–115. doi:10.3354/meps116099

Rogers AR, Harpending H (1992) Population-growth makes waves in the distribution of pairwise genetic-differences. Mol Biol Evol 9:552–569

Roman J, Palumbi SR (2004) A global invader at home: population structure of the green crab, *Carcinus maenas*, in Europe. Mol Ecol 13:2891–2898. doi:10.1111/j.1365-294X.2004.02255.x

Rousset F (1997) Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. Genetics 145:1219–1228

Saavedra C, Pena JB (2005) Nucleotide diversity and Pleistocene population expansion in Atlantic and Mediterranean scallops (*Pecten maximus* and *P-jacobaeus*) as revealed by the mitochondrial 16S ribosomal RNA gene. J Exp Mar Biol Ecol 323:138–150. doi:10.1016/j.jembe.2005.03.006

Shanks AL, Grantham BA, Carr MH (2003) Propagule dispersal distance and the size and spacing of marine reserves. Ecol Appl 13:S159–S169. doi:10.1890/1051-0761(2003)013[0159:PDDATS]2.0.CO;2

Shaw PW, Pierce GJ, Boyle PR (1999) Subtle population structuring within a highly vagile marine invertebrate, the veined squid *Loligo forbesi*, demonstrated with microsatellite DNA markers. Mol Ecol 8:407–417. doi:10.1046/j.1365-294X.1999.00588.x

Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. Genetics 139:1463 (vol 139, p 457, 1995)

Slatkin M, Excoffier L (1996) Testing for linkage disequilibrium in genotypic data using the expectation–maximization algorithm. Heredity 76:377–383. doi:10.1038/hdy.1996.55

Smith MJ, Arndt A, Gorski S, Fajber E (1993) The phylogeny of echinoderm classes based on mitochondrial gene arrangements. J Mol Evol 36:545–554. doi:10.1007/BF00556359

Stamatis C, Triantafyllidis A, Moutou KA, Mamuris Z (2004) Mitochondrial DNA variation in northeast Atlantic and Mediterranean populations of Norway lobster, *Nephrops norvegicus*. Mol Ecol 13:1377–1390. doi:10.1111/j.1365-294X.2004.02165.x

Stamatis C, Triantafyllidis A, Moutou KA, Mamuris Z (2006) Allozymic variation in Northeast Atlantic and Mediterranean populations of Norway lobster, *Nephrops norvegicus*. Mark Sci 63:875–882. doi:10.1016/j.icesjms.2006.01.006

Tajima F (1983) Evolutionary relationship of DNA-sequences in finite populations. Genetics 105:437–460

Tajima F (1989) The effect of change in population-size on DNA polymorphism. Genetics 123:597–601

Templeton AR, Crandall KA, Sing CF (1992) A cladistic-analysis of phenotypic Associations with Haplotypes Inferred from Restriction Endonuclease Mapping and DNA-Sequence Data. 3. Cladogram Estimation. Genetics 132:619–633

Templeton AR, Routman E, Phillips CA (1995) Separating population-structure from population history—a cladistic-analysis of the geographical-distribution of mitochondrial-DNA haplotypes in the Tiger Salamander, *Ambystoma-Tigrinum*. Genetics 140:767–782

Tintore J, Laviolette PE, Blade I, Cruzado A (1988) A study of an intense density front in the Eastern Alboran-Sea–the Almeria-Oran Front. J Phys Oceanogr 18:1384–1397. doi :10.1175/1520-0485(1988)018<1384:ASOAID>2.0.CO;2

Tortonese E (1980) Apérçu sommaire sur les asteroidea de la Méditerrannée (histoire, distribution, systematique). Journées d'études sur la systématique évolutive et la biogéographie en Méditerranée, Cagliari, pp 11–19

Triantafyllidis A, Apostolidis AP, Katsares V, Kelly E, Mercer J, Hughes M, Jorstad K, Tsolou A, Hynes R, Triantaphyllidis C (2005) Mitochondrial DNA variation in the European lobster (Homarus gammarus) throughout the range. Mar Biol (Berl) 146:223–235. doi:10.1007/s00227-004-1435-2

Turan C, Carvalho GR, Mork J (1998) Molecular genetic analysis of Atlanto-Scandian herring (*Clupea harengus*) populations using allozymes and mitochondrial DNA markers. J Mar Biol Assoc UK 78:269–283

Uthicke S, Benzie JAH (2003) Gene flow and population history in high dispersal marine invertebrates: mitochondrial DNA analysis of *Holothuria nobilis* (Echinodermata : Holothuroidea) populations from the Indo-Pacific. Mol Ecol 12:2635–2648. doi:10.1046/j.1365-294X.2003.01954.x

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538. doi:10.1111/j.1471-8286.2004.00684.x

Waters JM, O'Loughlin PM, Roy MS (2004) Cladogenesis in a starfish species complex from southern Australia: evidence for vicariant speciation? Mol Phylogenet Evol 32:236–245. doi:10.1016/j.ympev.2003.11.014

Waters JM, Roy MS (2004) Phylogeography of a high-dispersal New Zealand sea-star: does upwelling block gene-flow? Mol Ecol 13:2797–2806. doi:10.1111/j.1365-294X.2004.02282.x

Watterson GA (1984) Allele frequencies after a Bottleneck. Theor Popul Biol 26:387–407. doi:10.1016/0040-5809(84)90042-X

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population-structure. Evolution Int J Org Evolution 38:1358–1370. doi:10.2307/2408641

Williams ST (2000) Species boundaries in the starfish genus Linckia. Mar Biol (Berl) 136:137–148. doi:10.1007/s002270050016

Williams ST, Benzie JAH (1997) Indo-West Pacific patterns of genetic differentiation in the high-dispersal starfish Linckia laevigata. Mol Ecol 6:559–573. doi:10.1046/j.1365-294X.1997.00221.x

Williams ST, Benzie JAH (1998) Evidence of a biogeographic break between populations of a high dispersal starfish: congruent regions within the Indo-West Pacific defined by color morphs, mtDNA, and allozyme data. Evolution 52:87–99. doi:10.2307/2410923

Williams ST, Jara J, Gomez E, Knowlton N (2002) The marine Indo-West Pacific break: contrasting the resolving power of mitochondrial and nuclear genes. Integr Comp Biol 42:941–952. doi:10.1093/icb/42.5.941

Xia X, Xie Z (2001) DAMBE: Software package for data analysis in molecular biology and evolution. J Hered 92:371–373. doi:10.1093/jhered/92.4.371

Yasuda N, Nagai S, Hamaguchi M, Lian CL, Nadaoka K (2006) Development of microsatellite markers for the crown-of-thorns starfish *Acanthaster planci*. Mol Ecol Notes 6:141–143. doi:10.1111/j.1471-8286.2005.01168.x

Zane L, Ostellari L, Maccatrozzo L, Bargelloni L, Cuzin-Roudy J, Buchholz F, Patarnello T (2000) Genetic differentiation in a pelagic crustacean (*Meganyctiphanes norvegica* : Euphausiacea) from the North East Atlantic and the Mediterranean Sea. Mar Biol (Berl) 136:191–199. doi:10.1007/s002270050676

Zavodnik D (1960) Echinodermata der Insel Krk. Acta Adriat 9:3–19

Zhan AB, Bao ZM, Lu W, Hu XL, Peng W, Wang ML, Hu JJ (2007) Development and characterization of 45 novel microsatellite markers for sea cucumber (*Apostichopus japonicus*). Mol Ecol Notes 7:1345–1348. doi:10.1111/j.1471-8286.2007.01876.x

Zulliger D, Ruch M, Tanner S, Ribi G (2008) Characterization of nine microsatellite loci in the sea star *Astropecten aranciacus* and cross-species amplification for related taxa. Mol Ecol Res 8:634–636. doi:10.1111/j.1471-8286.2007.02027.x